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EFFECTS OF WATER CHEMISTRY ON AQUATIC
PLANTS: INTERACTIVE EFFECTS OF INORGANIC
CARBON AND NITROGEN ON BIOMASS
PRODUCTION AND PLANT NUTRITION

by

R. Michael Smart, John W. Barko

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199



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<p>Growth of the submersed aquatic plants <i>Egeria densa</i>, <i>Hydrilla verticillata</i>, and <i>Myriophyllum spicatum</i> was examined under two levels of inorganic carbon supply, at two sediment nitrogen (N) levels, in a solution containing moderate levels of major cations and inorganic carbon (CT). Levels of inorganic carbon supply were achieved by aeration with ambient air or CO₂-enriched air. Sediment N levels consisted of a control sediment and the same sediment after fertilization with ammonium chloride. The primary objective of the study was to evaluate the interactive effects of inorganic carbon supply and sediment N availability on plant growth and nutrition.</p> <p>Results indicate that both inorganic carbon supply and sediment N availability can limit the growth of submersed aquatic plant populations. Of the species studied here, <i>Egeria</i> was more affected by inorganic carbon supply, <i>Myriophyllum</i> was more affected by</p> <p style="text-align: right;">(Continued)</p>					
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Aquatic plants	<i>Hydrilla</i>	Nutrient uptake
Carbon limitation	<i>Myriophyllum</i>	Submersed macrophytes
<i>Egeria</i>	Nutrient limitation	Water chemistry

19. ABSTRACT (Continued).

sediment N availability, and *Hydrilla* was equally affected by both factors. Maximal plant growth of all species occurred under conditions of high inorganic carbon supply and high sediment N availability, indicating that suboptimal levels of either factor can depress growth. *Myriophyllum*, by virtue of its relatively greater root production, was more effective at N acquisition, and thus produced the most biomass under maximal growth conditions.

Increased N availability led to increased accumulation not only of N, but of other nutrients (phosphorus and potassium) as well. Under N-limiting conditions, increased N availability also promoted an increased demand for inorganic carbon, resulting in depletion of C_T from solution under the lower level of inorganic carbon supply. Depletion of solution C_T resulted from both enhanced photosynthetic rates and the resultant increased rates of $CaCO_3$ precipitation. Under inorganic carbon-limiting conditions, increased inorganic carbon availability increased the demand for both sediment and water nutrients.

These results suggest the existence of a feedback loop regulating biomass production in submersed aquatic plants. Both inorganic carbon and sediment nitrogen may limit biomass production in field populations. Increases in the supply of either limiting factor may result in increased demand for the other factor, eventually resulting in low levels of both factors. For this reason, biomass production in natural populations of submersed aquatic plants may decline over the years as both inorganic carbon supply and sediment N availability approach limiting levels.

Preface

The studies reported herein were sponsored by the Headquarters, US Army Corps of Engineers (HQUSACE), through the Aquatic Plant Control Research Program (APCRP). Funds were provided under Department of the Army Appropriation No. 96X3122, Construction General. Technical Monitor for the HQUSACE was Mr. James W. Wolcott. The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES) under the Environmental Resources Research and Assistance Programs, Mr. J. Lewis Decell, Manager.

Principal Investigator for this study was Dr. R. Michael Smart, Environmental Laboratory (EL), WES. The report was prepared by Dr. Smart with the assistance of Dr. John W. Barko. Technical assistance was provided by Ms. Dwilette G. McFarland. This report was reviewed by Drs. William Taylor and Kurt D. Getsinger, EL. The report was edited by Ms. Jessica S. Ruff of the WES Information Technology Laboratory.

This investigation was performed under the general supervision of Dr. John Harrison, Chief, EL, and Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division, and under the direct supervision of Dr. Thomas L. Hart, Chief, Aquatic Processes and Effects Group.

Commander and Director of WES was COL Larry B. Fulton, EN. Technical Director was Dr. Robert W. Whalin.

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EFFECTS OF WATER CHEMISTRY ON AQUATIC PLANTS:
INTERACTIVE EFFECTS OF INORGANIC CARBON AND NITROGEN
ON BIOMASS PRODUCTION AND PLANT NUTRITION

Introduction

1. This report is one of several describing the results of studies on the effects of water chemistry on submersed aquatic plants. Previous investigations of plant growth in relation to water chemistry (Smart and Barko 1986, 1988) have indicated that the water chemistry parameter of primary concern is dissolved inorganic carbon (C_T). Carbon, which accounts for more than 40 percent of the dry weight of aquatic plants (Sculthorpe 1967, Hutchinson 1975), generally occurs at levels less than those required to support maximal photosynthesis of submersed aquatic plants (Adams, Guilizzoni, and Adams 1978; Nichols and Shaw 1986; Smart and Barko 1986) and is thus potentially growth-limiting. Moreover, the simultaneous depletion of inorganic carbon and accumulation of oxygen that occurs within the boundary layer surrounding the leaves of submersed aquatic plants has required these plants to develop efficient mechanisms for assimilating inorganic carbon (Sand-Jensen 1989). In spite of the importance of inorganic carbon supply as a factor influencing species distribution and, potentially, biomass production, we have only a limited understanding of the interaction of the natural carbon regime with other environmental variables (Adams 1985).

2. In a previous report (Smart and Barko 1988) it was suggested that the growth of submersed aquatic plants was likely to be limited by either inorganic carbon supply or by sediment nitrogen (N) availability. Prior studies also indicated that photosynthesis associated with the growth of submersed aquatic plants causes significant changes in solution composition (Canfield, Maceina, and Shireman 1983; Smart and Barko 1986, 1988). These changes, which include reductions in dissolved inorganic carbon and calcium (Ca), occur more rapidly during periods of greatest plant growth (Smart and Barko 1988). If depletion of solution C_T is proportional to plant growth, factors affecting plant growth would be expected to affect inorganic carbon demand. Therefore, if the supply of N is growth-limiting, then increases in N availability may alter the response of plants to inorganic carbon availability. Likewise, if plant growth were limited by inorganic carbon availability, changes in this parameter would affect plant response to nitrogen. Therefore, in order to

understand the influence of either of these two important variables on plant growth, it is necessary to study their effects in combination as well as individually.

3. The objective of this study was to examine the interactive effects of inorganic carbon and nitrogen supply on the growth and nutrition of three species of submersed aquatic plants. In the experiment reported herein, we examined the responses of *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum* to inorganic carbon supply at each of two levels of sediment N availability.

Materials and Methods

4. The experiment was conducted in 150- by 90- by 90-cm-deep white fiberglass tanks in the greenhouse facility of the Environmental Laboratory located in Vicksburg, MS. Experimental treatments were randomly assigned to tanks in the greenhouse. Solutions were maintained at $25^{\circ} \pm 1^{\circ} \text{ C}$ with thermostatically controlled liquid circulators (Remcor Corporation, Chicago, IL). Gas exchange and mixing were facilitated by administering humidified, compressed air through twin air lifts in each tank. Experimental tanks were fitted with lucite covers to prevent the entry of dust and other contaminants. Neutral density shade fabric was used to reduce solar irradiance to maximal midday photosynthetically active radiation levels of about $750 \text{ uE m}^{-2} \text{ sec}^{-1}$.

5. Major cation concentrations and C_T levels in the experimental solution were based on analyses of bicarbonate lake waters (Hutchinson 1957). The solution used was a 50-percent dilution of the alkaline medium of Smart and Barko (1985) and was identical to that of the midlevel solution used in our earlier work (Smart and Barko 1988). The solution was formulated by additions of reagent grade chemicals to deionized (reverse osmosis) water. Gaseous CO_2 was administered to solution prior to adding required amounts of CaCO_3 to achieve solubility (Smart and Barko 1984, 1985). The chemical composition of the solution was verified by chemical analysis. Water samples were collected from each of the experimental tanks and analyzed weekly for Na, K, Ca, and Mg by atomic absorption spectrophotometry. Electrical conductivity (25° C) was determined with a YSI model 32 conductivity meter. Dissolved inorganic carbon was determined with a Beckman Model 915A total organic carbon analyzer. A Beckman Expandomatic IV expanded scale pH meter was used to measure pH.

Alkalinity and acidity were calculated from equations provided in Stumm and Morgan (1981).

6. Sediment used in the experiment was collected from Lake Washington, Washington, USA. This sediment was selected based on earlier investigations that indicated a high potential for supporting submersed macrophyte growth (Barko and Smart 1983, 1986). Physical and chemical characteristics of the sediment were similar to those provided earlier (Barko and Smart 1983, 1986). Sediment was stored at room temperature under anaerobic conditions until needed. The sediment was thoroughly mixed with a rotary mixer and then split into two aliquots for establishing the N availability treatment levels. The high N level was accomplished by adding NH_4Cl to one of the sediment aliquots during mixing at a rate of 0.2 g $\text{NH}_4\text{-N}/\ell$ sediment. The control sediment received no N addition. After mixing, the sediment was allocated to 1- ℓ sediment containers a few days prior to experimentation. The amount of N added to each container of the N-fertilized sediment was equivalent to that required to support a 20-g dry weight increase in shoot biomass.

7. Apical shoots, 15 cm in length, were taken from greenhouse cultures of *Egeria*, *Hydrilla*, and *Myriophyllum* that had been maintained at 25° C and at light levels similar to those to be used in the experiments. Four apices of a single species were randomly selected for planting in each of six replicate containers per treatment. After planting, a 2-cm layer of washed silica sand was placed over the sediment to minimize physical exchanges with the overlying water.

8. The experiment was conducted over a 6-week period between July and August. At termination of the experiment, plant shoots were clipped at the sediment surface, bagged, and dried at 80° C in a forced draft oven to constant weight. Roots were washed over a 1-mm sieve to remove sediment and debris, and dried as for shoots. Weights of root and shoot samples were recorded to the nearest milligram. Biomass attained by the end of the experimental period was considered to be indicative of plant growth for all species as, in every case, the initial biomass comprised less than 2-percent of final biomass.

9. Toward the end of the study, some of the plants of *Myriophyllum* and *Egeria* became very delicate and began to fragment. At the conclusion of the study all plant fragments were collected, dried, and weighed. Since fragmentation appeared to occur uniformly within a treatment, but was affected by

different treatments, we elected to correct the harvested biomass values to reflect the contribution of plant fragments.

10. Root and shoot samples were ground in a Wiley mill to pass a 40-mesh sieve. Subsamples of the dried and ground material were combusted in a muffle furnace at 550° C to determine ash content. Additional subsamples were digested in H₂O₂ - H₂SO₄ (Allen et al. 1974) and analyzed for total Kjeldahl nitrogen and total phosphorus on a Technicon Autoanalyzer. Major cations (Na, K, Ca, Mg) were determined on the same digestate by atomic absorption spectrophotometry.

11. The experiment included three species grown under two airstream CO₂ concentrations (ambient and 10 × ambient), at each of two sediment N levels (control sediment and the same sediment after N fertilization) in a 3 × 2 × 2 factorial design. Each of the treatment combinations was allocated to a single tank for a total of 12 tanks. Data were subjected to one-, two-, and three-way analysis of variance (ANOVA) procedures as appropriate (Statistical Analysis System, Cary, NC). Mean comparisons were performed using Duncan's Multiple Range Test and Dunnett's Procedure. Statistically significant differences referred to in the text were evaluated at the 5-percent (or less) level of statistical probability.

Results

Biomass production

12. Results of a three-way ANOVA (Table 1) indicate that total biomass production was significantly affected by all three main effects (species, carbon supply, and N availability). However, several interaction terms were also highly significant, indicating that the main effects were not independent. These significant interactions indicate that the main effects should be reexamined (Steel and Torrie 1960).

13. In an attempt to further clarify results, we reanalyzed the total biomass production data by species in a series of two-way ANOVAs (Table 2). The two-way ANOVAs indicate that total biomass production of all of the species was significantly affected by levels of both inorganic carbon supply and sediment N availability. However, the relative importance of these two factors differed among species. The growth of *Egeria* was affected more by inorganic carbon supply than by sediment N availability. The growth of *Hydrilla* was affected equivalently by each of these two factors. The growth

Table 1
Three-Way Analysis of Variance for Total Biomass Production

<u>Source</u>	<u>DF</u>	<u>ANOVA</u>		<u>Significance Level</u>
		<u>Sum of Squares</u>	<u>F Value</u>	
Species (Sp)	2	361.491	28.72	0.0001
Inorganic carbon (C)	1	760.435	120.82	0.0001
Sediment nitrogen (N)	1	823.030	130.76	0.0001
Interaction (Sp*C)	2	8.727	0.69	0.5039
Interaction (Sp*N)	2	148.879	11.83	0.0001
Interaction (C*N)	1	352.761	56.05	0.0001
Interaction (Sp*C*N)	2	19.110	1.52	0.2274

Table 2
Two-Way Analysis of Variance for Total Biomass Production

<u>Source</u>	<u>DF</u>	<u>ANOVA</u>		<u>Significance</u>
		<u>Sum of Squares</u>	<u>F Value</u>	<u>Level</u>
<i>Egeria densa</i>				
Inorganic carbon (C)	1	255.519	240.82	0.0001
Sediment nitrogen (N)	1	71.519	67.40	0.0001
Interaction (C*N)	1	68.310	64.38	0.0001
<i>Hydrilla verticillata</i>				
Inorganic carbon (C)	1	190.463	15.97	0.0007
Sediment nitrogen (N)	1	243.398	20.41	0.0002
Interaction (C*N)	1	99.919	8.38	0.0090
<i>Myriophyllum spicatum</i>				
Inorganic carbon (C)	1	323.180	54.84	0.0001
Sediment nitrogen (N)	1	656.993	111.48	0.0001
Interaction (C*N)	1	203.642	34.55	0.0001

of *Myriophyllum* was affected more by sediment N availability than by inorganic carbon supply. The highly significant interaction terms again indicate that the responses of the plants to carbon and nitrogen were not independent.

14. To examine the physiological significance of the treatment interactions, we further analyzed the data according to a one-way classification. In this analysis we considered the ambient air aeration-unfertilized sediment treatment combination as a control. Each of the other treatment combinations was then compared with the control. This analysis allowed us to independently assess the significance of increasing the level of each of the treatment variables, both singly and in combination.

15. In comparison with the control condition, biomass production by *Egeria* responded to an increase in CO_2 supply but not to an increase in sediment N (Figure 1). Growth of this species under control conditions was thus

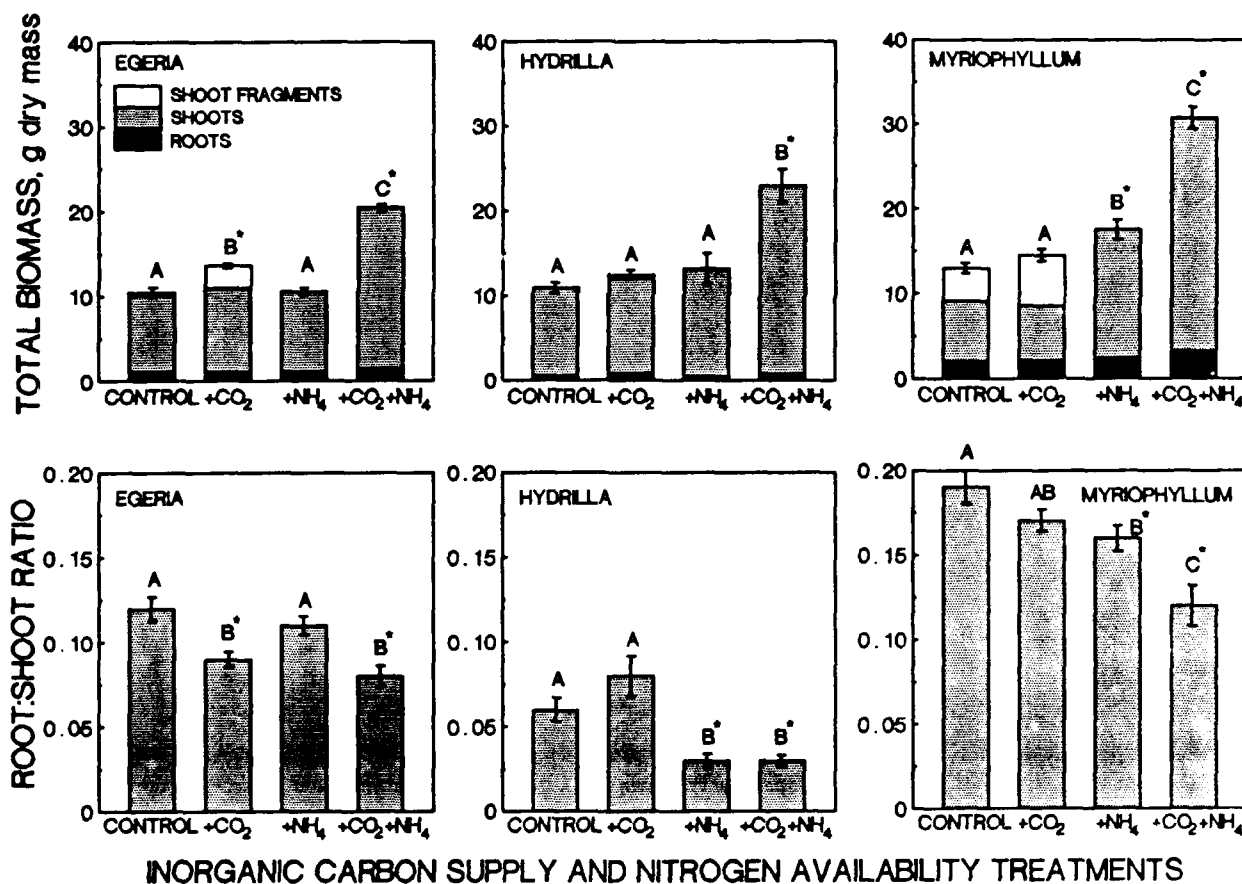


Figure 1. Total biomass and root:shoot biomass ratio of *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum*, in relation to inorganic carbon supply and sediment nitrogen availability. Values are means \pm standard error of the mean based on six replications. Bars sharing the same letter do not differ at the 5-percent level of statistical significance. Bars marked with an asterisk differ significantly from the control

carbon-limited. However, increasing the level of airstream CO₂ increased biomass production and resulted in subsequent N-limitation in the high-CO₂ treatment, as evidenced by the significance of the total biomass response to N at high CO₂ supply.

16. Biomass production of *Hydrilla* did not respond to an increased supply or either CO₂ or N alone (Figure 1). From the lack of response to independent addition of either CO₂ or N it would seem that the growth of this species was not limited by either of these elements. However, simultaneous additions of both CO₂ and N promoted a dramatic increase in biomass production, indicating that the growth of *Hydrilla* under control conditions was limited by both inorganic carbon and nitrogen.

17. Biomass production of *Myriophyllum* responded to addition of N but not to increased CO₂ supply (Figure 1). Growth of this species under the control condition was thus considered to be N-limited. Increasing N availability increased biomass production and resulted in subsequent carbon limitation in the N-fertilized treatment, as evidenced by the significance of the total biomass response to CO₂ supply in the high N treatments.

18. Maximal biomass production of all three species occurred under the combination of high CO₂ supply and high sediment N availability. This indicates that maximal growth of all three species requires high levels of both inorganic carbon and nitrogen. Moreover, growth of all of the species was N-limited under the high level of inorganic carbon supply and was carbon-limited under the high level of sediment N availability. Thus, even though the individual species responded somewhat differently to independent additions of each of the treatment variables, growth of all the species was similarly affected by the combination of the two variables.

19. Root biomass production in *Myriophyllum* was significantly greater than in the other two species. This high level of root biomass results in a relatively high root:shoot biomass ratio for *Myriophyllum* (Figure 1). The relatively high root:shoot ratio of *Myriophyllum* has been noted earlier (Barko and Smart 1986). Typically high root:shoot ratios are considered to be of adaptive value in infertile environments. The adaptive significance of high root:shoot ratios may involve a relatively greater ability to exploit sediment nutrients, a greater ability to store accumulated nutrients, or both.

20. Root:shoot ratios in *Hydrilla* and *Myriophyllum* were lower in plants grown at the higher level of N availability. This response of decreasing root:shoot ratios with increasing sediment fertility is typical of plants in

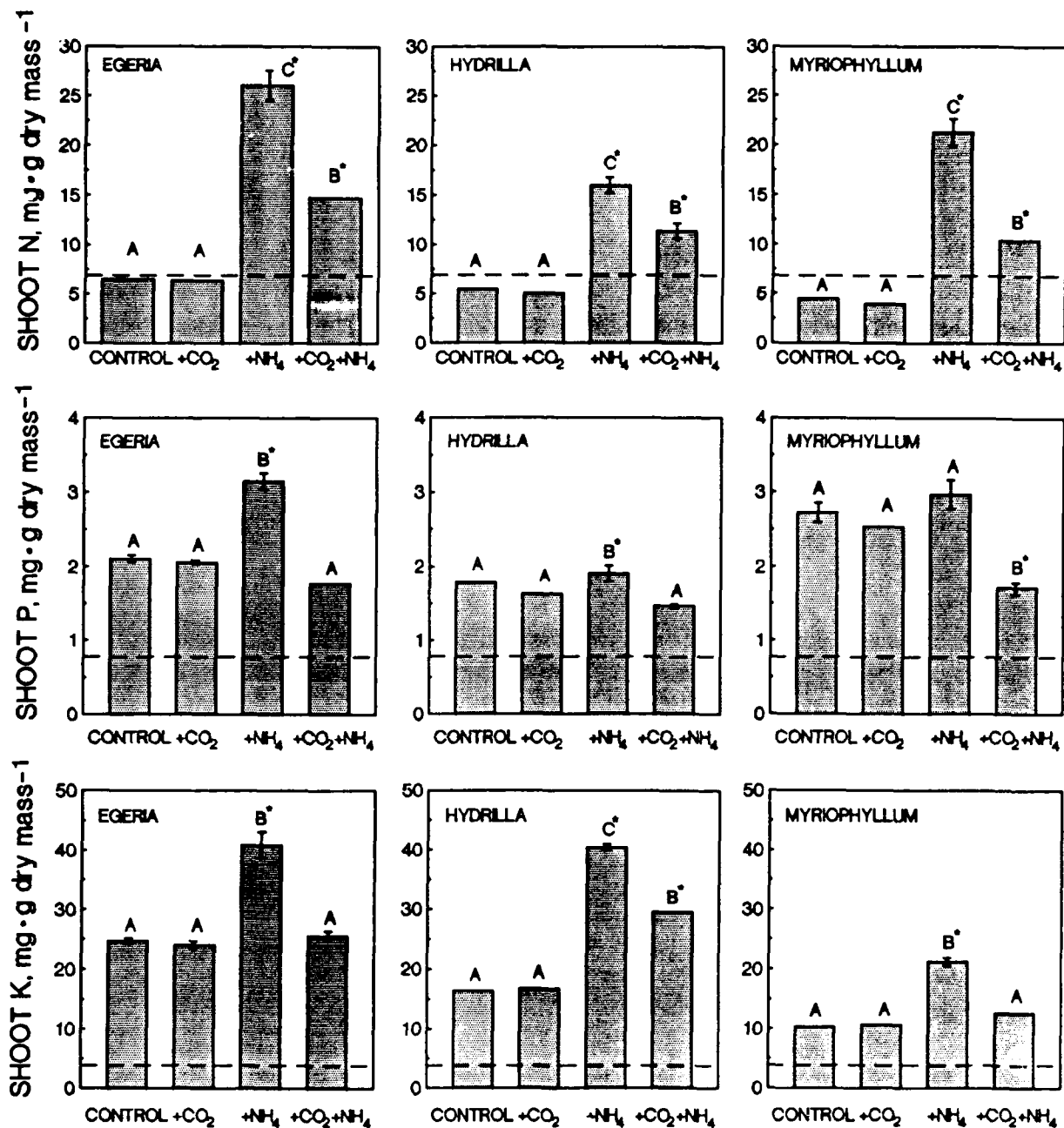
general, and submersed aquatic plants in particular (Denny 1972, Barko and Smart 1986). It is interesting that the root:shoot ratio in *Hydrilla* was responsive to the addition of N alone even though total biomass production was not. In contrast with the other two species, root:shoot ratio in *Egeria* was affected more by inorganic carbon supply than by sediment N availability. This result parallels the growth response of this species, which was more affected by inorganic carbon supply than by N availability.

21. Shoot fragmentation was particularly acute in plants grown under increased CO₂ supply at the control level of N availability (Figure 1). Fragmentation did not occur to any significant extent in *Hydrilla*, or in any of the plants grown under conditions of increased N availability. Fragmentation may thus represent a plant response to N limitation, at least in *Egeria* and *Myriophyllum*. Fragmentation may be useful as an index of nutrient stress in these species.

22. On the control sediment, all three species produced similar quantities of shoot and total biomass; however, on the N-fertilized sediment, *Myriophyllum* produced significantly more shoot and total biomass than did the other two species. The high level of root production in this species may have contributed to its greater ability to respond to increased sediment N availability.

Plant nutrition

23. Shoot nitrogen concentrations measured at the end of the study (Figure 2) were below critical levels in plants of all species grown on control sediment. In contrast, shoot N concentrations of plants grown on the N-fertilized sediment were above critical concentrations for each of the species, indicating that N fertilization was sufficient to satisfy the demand for N. Shoot nutrient concentrations at or below the critical level are considered indicative of nutrient limitation (Gerloff 1975, Barko and Smart 1986). Thus, while biomass production of *Hydrilla* and *Egeria* was not responsive to additions of N alone, tissue analysis suggests that the growth of all three species on the control sediment was N-limited. However, tissue analysis obtained from a single harvest (at the completion of the experiment) is indicative only of nutrient status at that particular time, while biomass production integrates the effects of nutritional status over the entire growth period. Tissue analysis as employed here is, therefore, only indicative of nutritional status at the conclusion of the study period. Thus, N limitation may have occurred only near the end of the experimental period, and *Egeria* and



INORGANIC CARBON SUPPLY AND NITROGEN AVAILABILITY TREATMENTS

Figure 2. Shoot concentrations of nitrogen, phosphorus, and potassium in *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum*, in relation to inorganic carbon supply and sediment nitrogen availability. Dashed lines indicate generalized critical concentrations. Values are means \pm standard error of the mean based on three replications. Bars sharing the same letter do not differ at the 5-percent level of statistical significance. Bars marked with an asterisk differ significantly from the control

Hydrilla may not have been subjected to N-limiting conditions for a sufficient period of time to significantly affect biomass production. Barko et al. (1988) presented temporal tissue analysis data indicating that the growth of *Hydrilla* on the same Lake Washington sediment was not limited by N during the first 4 to 6 weeks of the growth period, and that N limitation likely occurred between 6 and 8 weeks.

24. Highest shoot N concentrations occurred under the combination of ambient air aeration and sediment N fertilization. Biomass production of all three species was limited by inorganic carbon supply under this set of conditions; thus, the plants were unable to use all of the acquired N in the production of additional biomass. Increased inorganic carbon availability increased biomass production of all species grown on the N-fertilized sediment, resulting in a reduction of shoot N in these plants relative to those receiving N alone.

25. Shoot P and K concentrations of all species were above respective critical concentrations of these elements (Figure 2) under all of the growth conditions. Thus, P and K were not involved in growth limitation. Increased CO₂ supply alone had no effect on shoot concentrations of N, P, or K of plants grown on the control sediment. However, fertilization with N generally resulted in elevated shoot concentrations of P and K as well as of N. Increasing carbon availability to plants grown on the N-fertilized sediment resulted in a reduction of shoot concentrations of P and K as well as N in these plants relative to those receiving ambient air aeration. The increased biomass production of plants receiving both CO₂ and N additions likely resulted in the reduced shoot concentrations of these elements relative to those in plants receiving N alone.

26. The increase in shoot concentrations of N, P, and K resulting from N fertilization suggests that the uptake of these elements is closely coupled. Barko and Smart (1986) previously demonstrated that nutrient stress in *Hydrilla* and *Myriophyllum* resulting from growth on infertile sediments reduced shoot concentrations of N, P, and K as well as iron. In another investigation, Barko et al. (1988) indicated that N limitation in *Hydrilla* resulted in depressed shoot concentrations of P and K, as well as nitrogen. In the investigation reported here, we further demonstrate that augmenting the supply of a limiting nutrient stimulates uptake of other, nonlimiting nutrients. This stimulation, resulting in "luxury consumption" of other nutrients, may be of

adaptive value for plants growing in the environments characterized by episodic nutrient loadings.

Nutrient accumulation

27. Shoot nutrient accumulation (the product of shoot biomass and shoot nutrient concentration) was more affected by N availability than by inorganic carbon supply (Figure 3). Shoot N accumulation was affected by CO₂ supply only in *Egeria*. Even though biomass production of *Egeria* and *Hydrilla* was not responsive to additions of N alone, N fertilization resulted in large increases in shoot N accumulation in these species as well as in *Myriophyllum*. Nitrogen fertilization, irrespective of the individual species' biomass responses to N, caused roughly equivalent increases in shoot N accumulation.

28. The increase in shoot N accumulation by plants grown on the N-fertilized sediment in comparison to the control sediment accounts for much of the N added to the sediment. Of the 200 mg N added, 84 percent can be accounted for by the increase in N of *Hydrilla* shoots, 96 percent in *Egeria* shoots, and 129 percent in *Myriophyllum* shoots (Table 3). These recoveries parallel similar trends in increasing root biomass and root:shoot biomass ratio. The very high recovery of added N in *Myriophyllum* shoots, in addition to the large biomass response of this species to added N, indicates that the well-developed root system of *Myriophyllum* is quite efficient in nutrient acquisition.

29. Shoot accumulations of P and K were also affected by N fertilization, irrespective of the individual species' biomass responses to N. Shoot accumulations of P and K in *Egeria* also increased in response to increased inorganic carbon supply to plants grown on both control and N-fertilized sediments (Figure 3). In the case of the former, these increased shoot nutrient accumulations are attributable to an increase in shoot biomass production, as shoot concentrations of these elements were unaffected by CO₂ supply to plants grown on control sediment. This result corroborates the earlier observation by Barko and Smart (1986), that increased biomass production accompanying an increase in availability of the limiting resource can result in an increased demand for nutrients. This occurrence may have important consequences for plant growth over the long term.

30. On the control sediment, the three species accumulated similar quantities of shoot nitrogen; however, on the N-fertilized sediment, *Myriophyllum* accumulated significantly more N (and P as well) than did the other species. Again, this may have resulted from the ability of *Myriophyllum* to

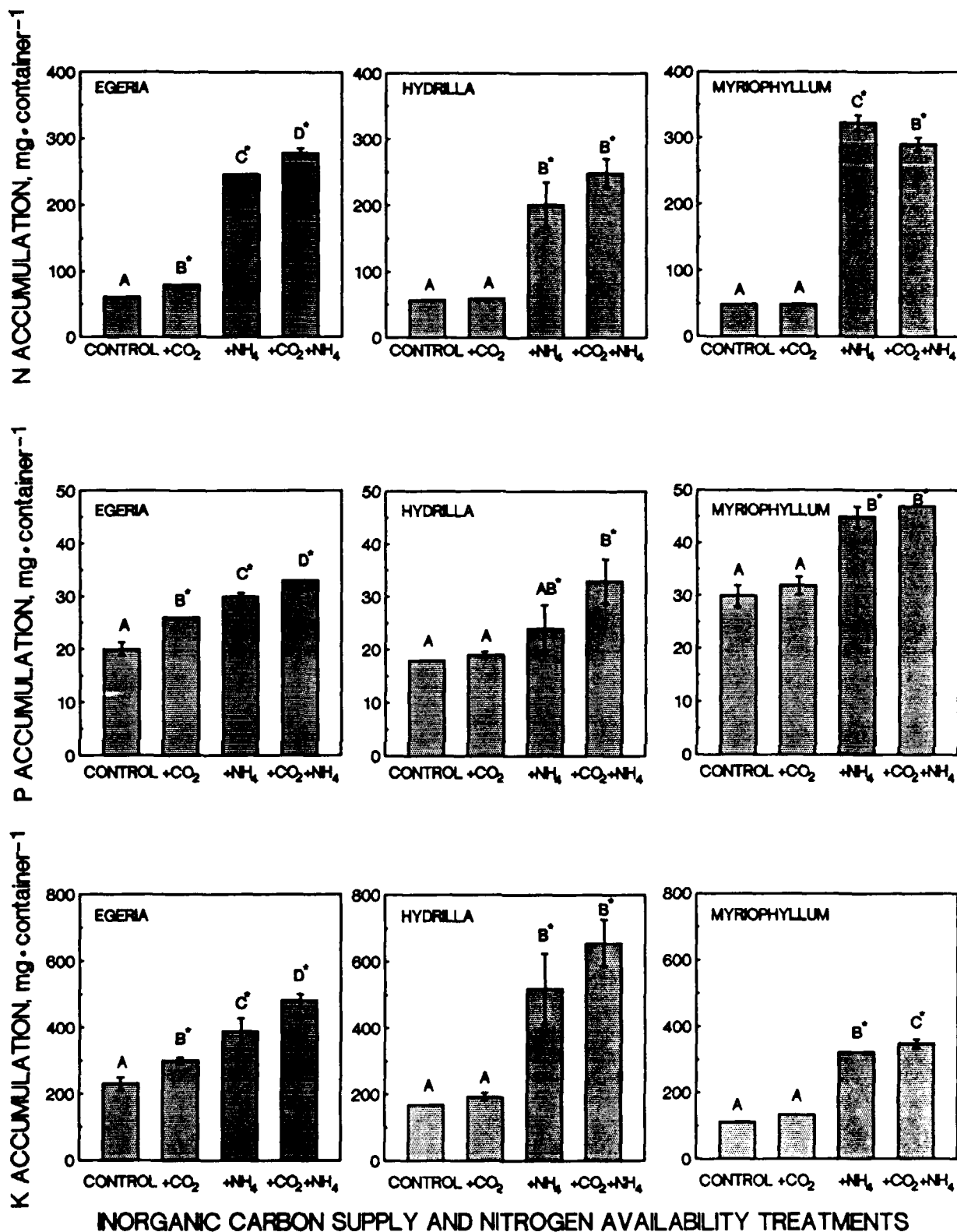


Figure 3. Accumulations of nitrogen, phosphorus, and potassium in shoots of *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum*, in relation to inorganic carbon supply and sediment nitrogen availability. Values are means \pm standard error of the mean based on three replications. Bars sharing the same letter do not differ at the 5-percent level of statistical significance. Bars marked with an asterisk differ significantly from the control

Table 3
Shoot Nitrogen Accumulation (mg container⁻¹) and Percent
 Recovery of Added Nitrogen* in Shoot Biomass

Treatment	Species		
	<i>Egeria</i>	<i>Hydrilla</i>	<i>Myriophyllum</i>
+NH ₄	246	201	323
Control	<u>61</u>	<u>57</u>	<u>49</u>
Difference	185	144	274
Percent recovery	93	72	137
+CO ₂ +NH ₄	278	249	291
+CO ₂	<u>80</u>	<u>58</u>	<u>49</u>
Difference	198	191	242
Percent recovery	99	96	121
Mean difference	192	168	258
Mean percent recovery	96	84	129

* 200 mg N added to +NH₄ sediments.

produce more roots than either *Egeria* or *Hydrilla* (Figure 1). However, K accumulation in *Myriophyllum* was less than in *Egeria* and *Hydrilla* under all experimental conditions. Unlike N and P, which are acquired by roots from sediment (Barko and Smart 1980, 1981), K is generally acquired by foliar uptake from the water column (Barko 1982, Huebert and Gorham 1983, Smart and Barko 1985). These findings suggest that *Myriophyllum*, by virtue of its greater root development, may be more capable of exploiting highly eutrophic conditions than *Egeria* and *Hydrilla*.

Solution composition

31. Under the control condition, both *Egeria* and *Hydrilla*, which were not limited by N, exerted significant demands on C_T, as evidenced by the depletion of C_T from solution (Figure 4). In contrast, *Myriophyllum*, which was limited by N under the control condition, did not promote a substantial reduction in solution C_T. Addition of N to sediment, under ambient air aeration, promoted increased biomass production in *Myriophyllum*, resulting in an increased demand for inorganic carbon and a significant depletion of solution C_T under this condition. In contrast, addition of N to sediment did not promote increased biomass production in *Egeria*, which was limited by inorganic carbon under the control condition. Thus, C_T depletion in *Egeria* under

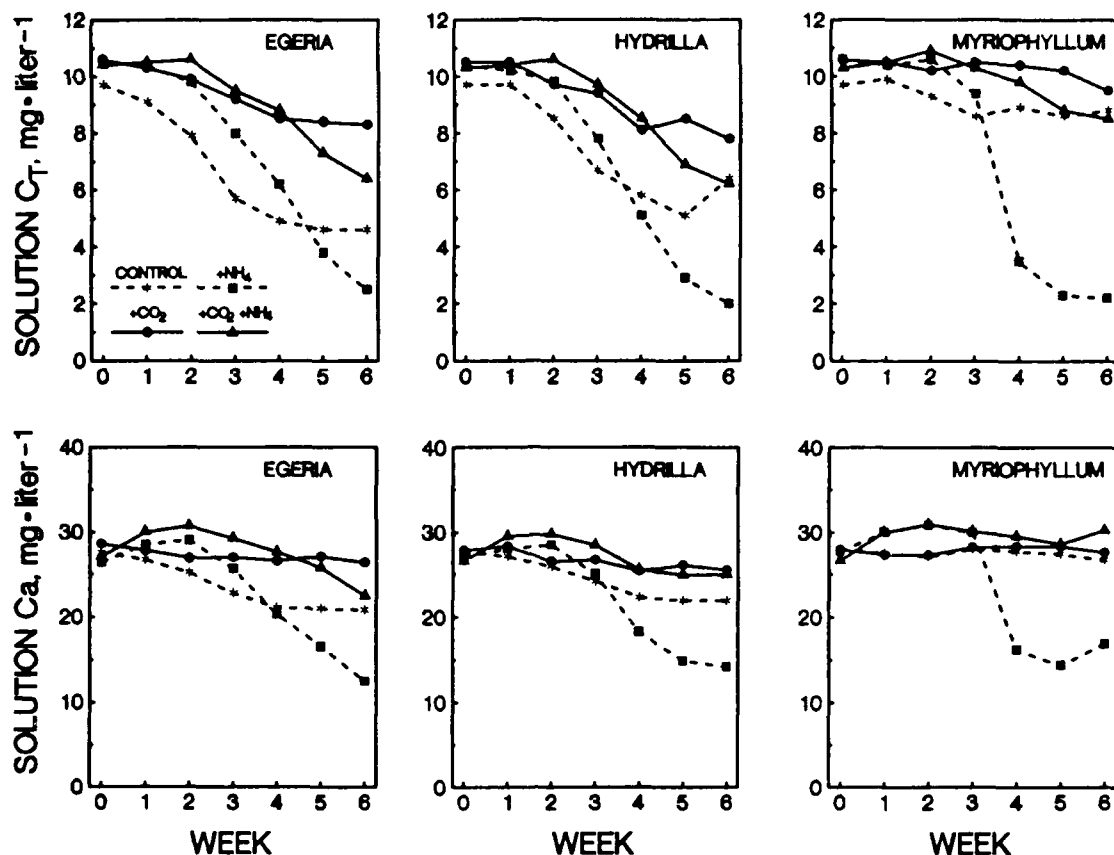


Figure 4. Temporal changes in dissolved inorganic carbon (C_T) and dissolved calcium in experimental solutions containing *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum*, in relation to inorganic carbon supply and sediment nitrogen availability

ambient air aeration did not differ greatly between plants grown on the control and N-fertilized sediments. Likewise, addition of N to sediment did not promote increased biomass production in *Hydrilla*; however, *Hydrilla* may have been limited by N during the latter few weeks of the experiment. During this period, N-fertilized *Hydrilla* exerted an increased demand for inorganic carbon as compared with control plants, resulting in a reduction of solution C_T to a very low level.

32. The 10-fold increase in CO_2 supply was moderately effective at maintaining solution C_T at levels near the original amount for plants grown on control sediment. Under the increased plant demand for inorganic carbon promoted by N fertilization, the higher level of CO_2 supply was less effective at maintaining original levels of C_T , particularly in solutions supporting the growth of *Egeria* and *Hydrilla*. Increased biomass production resulting from N

fertilization thus can result in an increased demand for inorganic carbon as well as for P and K.

33. Changes in solution Ca during the study reflect changes in solution C_T (Figure 4). The similarity in the responses of Ca and C_T indicate that changes in these parameters are due to both photosynthesis and CaCO_3 precipitation, as has been demonstrated earlier for this solution (Smart and Barko 1988). Like C_T , Ca was most affected by plant growth under the combination of low CO_2 supply and high sediment N availability. Also like C_T , changes in Ca were moderated by the 10-fold increase in CO_2 supply, indicating that addition of CO_2 decreased the rate of CaCO_3 precipitation. This result was not unexpected, as increases in CO_2 cause a shift in the carbonate equilibrium away from the formation of CO_3 (Stumm and Morgan 1981).

34. Since CaCO_3 precipitation in addition to photosynthesis is stimulated by increased N availability, short-term increases in N may result in long-term decreases in solution C_T . This interaction between these two potentially growth-limiting elements may play an important role in regulating plant biomass production.

35. As in the previous report in this series (Smart and Barko 1988), growth of all species caused significant reductions in solution K (paired comparisons t-test). Changes in solution K ranged from 0.6 to 3.0 mg l^{-1} and were directly proportional to shoot accumulations of this element (Figure 5). This result suggests that changes in solution K resulted from foliar uptake. While the previous study (Smart and Barko 1988) indicated that K uptake from solution can exceed K accumulation in shoot tissues, K uptake and accumulation in this study did not differ consistently (paired comparisons t-test). The exchange of foliarly derived K with the sediment exchange complex has been suggested as a mechanism for coping with low N availability, at least in *Hydrilla* (Barko et al. 1988). If excessive K uptake (and subsequent translocation and exchange) is somehow involved in N nutrition, the increase in sediment N availability in this study may have been responsible for the discrepancy between the two experiments.

36. The previous study indicated that solution K depletion was proportional to plant biomass production (Smart and Barko 1988). The results of the present study substantiate this and also indicate that the demand for solution K is affected by N availability. This result indicates that increased sediment N availability not only promotes increased uptake of N and P from the sediment, but also increases uptake of K from the water column. Nitrogen thus

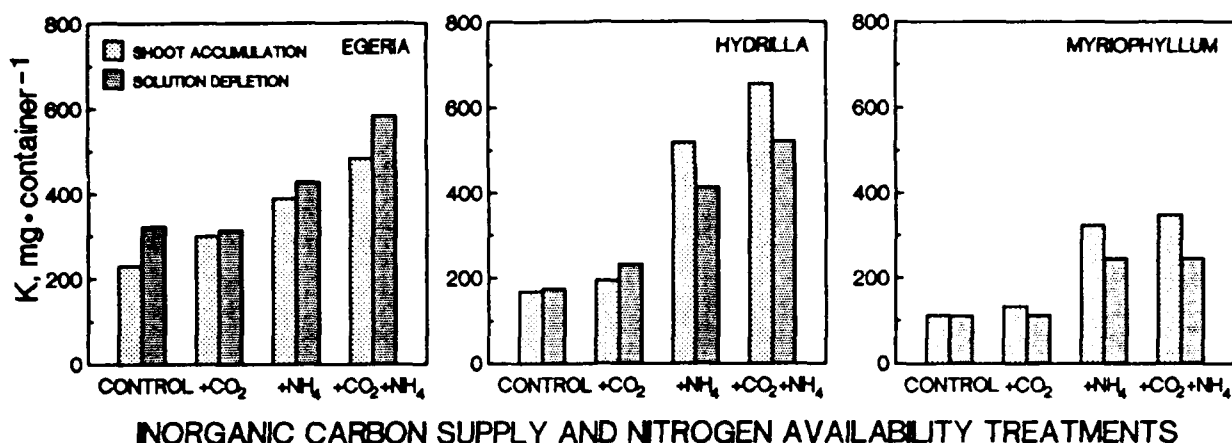


Figure 5. Shoot K accumulation by *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum* and solution K depletion by the same species in relation to inorganic carbon supply and sediment nitrogen availability. Values for shoot K accumulation (light bars) are means ($n = 3$) of the product of shoot mass and shoot K concentration. Values for solution K depletion (dark bars) were determined from differences in initial and final solution K concentrations expressed on a per container basis

seems to be a master variable, affecting plant demand for other nutrient elements as well as for inorganic carbon.

Discussion

37. Prior studies have indicated that, given adequate light levels and temperatures, the growth of submersed aquatic plants is likely to be limited by the supply of inorganic carbon (Hough and Fornwall 1988) or by the availability of sediment nitrogen (Smart and Barko 1988). Of the three species studied here, *Egeria* was more responsive to inorganic carbon supply than to sediment N availability, *Myriophyllum* was more responsive to sediment N, and *Hydrilla* was equally responsive to both factors. Biomass production of all three species was greatly enhanced by increasing the supply of both of these important elements. This result suggests that maintenance of problem levels of submersed aquatic plant biomass requires high levels of both inorganic carbon supply and sediment nitrogen availability. Reductions in either of these factors may be sufficient to alleviate the severity of aquatic plant infestations.

38. Increases in the supply of a limiting resource result in increased biomass production, which results in an increased demand on other (potentially limiting) resources. For this reason, short-term excesses in biomass

production may actually accelerate long-term reductions in biomass production. For example, a previously uncolonized sediment may contain high levels of available N and may initially support a high level of biomass production. This high biomass production rate places a high demand on the rate of supply of inorganic carbon, resulting in depletion of C_T and subsequent carbon-limited growth. Alternatively, a high rate of supply of inorganic carbon results in a high rate of biomass production. This high rate of biomass production causes an increased demand for sediment N, eventually reducing N availability to levels insufficient to support problem levels of plant biomass.

39. While the demand for inorganic carbon fluctuates diurnally with photosynthesis, the demand for N is more seasonal. The requirement for inorganic carbon is also more immediate in that most species of submersed aquatic plants cannot store appreciable quantities of carbon to be used during later periods of high demand. Submersed plants can, however, store appreciable quantities of N to meet later demands for growth. For these reasons, inorganic carbon supply can be important in regulating photosynthesis and short-term growth rates, while N availability is perhaps more important in setting upper limits on potential biomass attainment. If the supply of inorganic carbon is plentiful, the attainment of maximum potential biomass will be accomplished more rapidly. Since sediment N availability decreases due to autogenic processes as plant populations age (Barko et al. 1988), factors accelerating biomass production are likely to accelerate autogenic reductions in N availability. For this reason, high levels of inorganic carbon availability may actually accelerate the decline of submersed aquatic plant biomass as populations age, allowing ecosystem succession to proceed.

40. High levels of both of these factors will stimulate increased growth, increasing the demand on the other factor, until one of them (or some other environmental factor) becomes growth-limiting. Long-term biomass production in these populations may thus be self-regulating. This reciprocal relationship between inorganic carbon supply and sediment N availability is an example of an interactive feedback control system that may be involved in regulating the growth of rooted submersed aquatic plants. If this control system does in fact operate in natural systems, the production of very high levels of aquatic plant biomass may require both a high rate of inorganic carbon supply and high sediment N availability. Reductions in either of these factors could result in reduced rates of plant growth. Indirect evidence for

this occurrence is provided by the cycle of explosive growth followed by decline, which is characteristic of many species of submersed aquatic plants. Additional circumstantial evidence is provided by the decline of *M. spicatum* in Lake Wingra corresponding with a 31-percent reduction in C_T levels in that lake (Titus and Stone 1982).

Conclusions and Recommendations

41. Photosynthesis and growth are strongly dependent on the supply of inorganic carbon, and, under conditions of adequate light, temperature, and sediment nutrient availability, carbon limitation of submersed aquatic plant populations is likely. Under natural environmental conditions, biomass production of submersed plant populations is likely to be limited by the rate of supply of either inorganic carbon or N. The difference between the occurrence of innocuous submersed aquatic plant populations and the development of noxious aquatic "weed" infestations may depend on the relative rates of supply of carbon and/or N to field populations.

42. It is recommended that future investigations consider the interactive roles of inorganic carbon supply and N availability in controlling biomass production of submersed aquatic plant populations. These investigations would be most profitably conducted under field conditions in order to determine longer term responses of plant populations to environmental conditions. Results of these types of studies might reveal methods of managing the environment to minimize biomass production of submersed aquatic plants.

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